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ORIGINAL REPORT

Low Serum Vitamin D Levels Are Associated With Inferior Survival in Follicular Lymphoma: A Prospective Evaluation in SWOG and LYSA Studies

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ABSTRACT

Purpose
Recent literature reports a potential association between high vitamin D and improved lymphoma prognosis. We evaluated the impact of pretreatment vitamin D on follicular lymphoma (FL) outcome.

Patients and Methods
SWOG participants were previously untreated patients with FL enrolled onto SWOG clinical trials (S9800, S9911, or S0016) involving CHOP chemotherapy plus an anti-CD20 antibody (rituximab or iodine-131 tositumomab) between 1998 and 2008. Participants included in our second independent cohort were also previously untreated patients with FL enrolled onto the Lymphoma Study Association (LYSA) PRIMA trial of rituximab plus chemotherapy (randomly assigned to rituximab maintenance *v* observation) between 2004 and 2007. Using the gold-standard liquid chromatography–tandem mass spectrometry method, 25-hydroxyvitamin D was measured in stored baseline serum samples. The primary end point was progression-free survival (PFS).

Results
After a median follow-up of 5.4 years, the adjusted PFS and overall survival hazard ratios for the SWOG cohort were 1.97 (95% CI, 1.10 to 3.53) and 4.16 (95% CI, 1.66 to 10.44), respectively, for those who were vitamin D deficient (< 20 ng/mL; 15% of cohort). After a median follow-up of 6.6 years, the adjusted PFS and overall survival hazard ratios for the LYSA cohort were 1.50 (95% CI, 0.93 to 2.42) and 1.92 (95% CI, 0.72 to 5.13), respectively, for those who were vitamin D deficient (< 10 ng/mL; 25% of cohort).

Conclusion
Although statistical significance was not reached in the LYSA cohort, the consistent estimates of association between low vitamin D levels and FL outcomes in two independent cohorts suggests that serum vitamin D might be the first potentially modifiable factor to be associated with FL survival. Further investigation is needed to determine the effects of vitamin D supplementation in this clinical setting.

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INTRODUCTION

Follicular lymphoma (FL) is the second most common subtype of non-Hodgkin lymphoma. Although outcomes have improved substantially in the modern therapeutic era, FL is still characterized by a generally incurable clinical course. FL prognosis is known to be influenced by clinical characteristics and age; however, investigation of modifiable prognostic and predictive factors in the modern treatment era has been limited.

Since a link between solar radiation, vitamin D production, and decreased colon cancer mortality was established in 1980, animal and human research has been ongoing to investigate the association between vitamin D status and many cancers.[1] Recent published evidence supports a survival benefit with higher vitamin D levels in multiple malignancies.[2] Several recent studies have suggested that increased sun exposure (primary vitamin D source) is protective against lymphoma, although the literature to date is limited with regard to an association between vitamin D status and lymphoma risk.[3] However, evidence of a biologic effect of 1,25-dihydroxyvitamin D on lymphoma progression has been demonstrated in the laboratory, with observed promotion of differentiation and antiproliferative effects on lymphoma cell lines in vitro.[4,5] Moreover, survival benefit with vitamin D sufficiency among patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL)[6,7] and chronic lymphocytic leukemia[8] has been recently reported.

We therefore hypothesized that patients with FL with insufficient vitamin D would have inferior outcomes. The primary aim of this analysis was to evaluate the role of pretreatment serum 25-hydroxyvitamin D [25(OH)D] with regard to progression-free survival (PFS) among two independent cohorts of similarly treated prospective patients with newly diagnosed FL.

PATIENTS AND METHODS

This secondary observational analysis was reviewed by the University of Rochester Institutional Review Board and was registered with ClinicalTrials.gov.

Study Populations

SWOG cohort. Newly diagnosed, previously untreated patients with FL (stage III or IV or bulky II disease) enrolled onto one of three SWOG clinical trials involving CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy plus an anti-CD20 antibody were eligible for inclusion in this cohort: S9800,[9] S9911,[10] and S0016.[11,12] Eligibility criteria for these three studies enrolling patients with biopsy-proven, untreated FL were identical and previously described.[9-12] Patients enrolled onto any of these three trials who also had pretreatment serum stored and available through the SWOG serum banking protocol (S8947) were eligible for this analysis. Patients were observed for progression with clinical examination and computed tomography scan (3 months during treatment, every 6 months for 2 years after therapy, and annually thereafter) using guidelines from two international workshops.[13,14]

LYSA cohort. Patients included in our second independent cohort also had biopsy-confirmed, previously untreated FL (grade 1, 2, or 3a) and were enrolled onto the Lymphoma Study Association (LYSA; formerly Groupe d'Étude des Lymphomes de l'Adulte) PRIMA (Primary Rituximab and Maintenance) trial[15] of rituximab plus chemotherapy (randomly assigned to rituximab maintenance *v* observation) between 2004 and 2007, as previously described,[15] and had pretreatment serum samples stored and available for serum 25(OH)D analysis. Only those who were treated with R-CHOP (rituximab plus CHOP) induction were eligible for inclusion in the LYSA cohort for this analysis. Patients who were registered before induction treatment but not randomly assigned (rituximab maintenance *v* no maintenance therapy) after R-CHOP were included in this analysis. Patients were actively observed for progression with clinical examination (every 8 weeks for first 2 years after induction; every 3 months for additional 3 years) and computed tomography scan (every 6 months for first 2 years after induction; every 6 months for additional 3 years). Clinical response to R-CHOP induction was assessed 2 to 4 weeks after the final R-CHOP cycle.[13]

Vitamin D Measurement

Vitamin D level was determined from a single baseline serum sample. All samples (from both SWOG and LYSA cohorts) were sent to the Mayo Clinic Medical Laboratories,[16] where 25(OH)D_2 and 25(OH)D_3 were measured directly using the gold-standard[17] liquid chromatography–tandem mass spectrometry (LC-MS/MS) method, and the 25(OH)D value used throughout our analyses was the total 25(OH)D value [ie, sum of 25(OH)D_2 and 25(OH)D_3]. Measurements of serum vitamin D were obtained by putting the specimens on protein crash plates (Chrom Tech, Apple Valley, MN). A positive pressure manifold transferred the supernatant to a collection plate, which was put onto a Sciex API 4000 Qtrap Multiplex HPLC system (AB SCIEX, Framingham, MA), using Shimadzu pumps (Kyoto, Japan) and atmospheric pressure chemical ionization source. To control for variability in 25(OH)D levels by season and latitude, we included calendar year quarter of baseline blood draw and latitude (stratified at 35°N[18] in SWOG cohort; Europe *v* Australia in PRIMA cohort) in Cox proportional hazards models. We also ran a series of seasonal adjustment sensitivity analyses, considering continuous adjustment for quarter, using season-specific 25(OH)D quartiles[19] and continuous rank statistics with adjustment for quarter.

Vitamin D Deficiency

A recent report by the US Institute of Medicine recommended serum 25(OH)D ≥ 20 ng/mL to maximize bone health.[20] Accordingly, 25(OH)D was evaluated as a dichotomous variable (deficient, < 20 *v* sufficient, ≥ 20 ng/mL) for the SWOG cohort. Outside of the United States, a global definition of vitamin D deficiency has commonly been serum 25(OH)D < 10 ng/mL,[21] and a threshold of 8 ng/mL was recently used in a German study of DLBCL and prognosis.[7] As such, the threshold used for defining vitamin D deficiency in the LYSA cohort was serum 25(OH)D < 10 ng/mL.

Statistical Analysis

Standard summary statistics were used to describe each study population in terms of clinical and demographic characteristics, and χ^2 tests were used to evaluate the association between these baseline characteristics and vitamin D deficiency. Our primary end point was PFS, defined as time from date of enrollment or registration to date of progression or death resulting from any cause. We evaluated serum 25(OH)D as a dichotomous variable and compared PFS between the patients who were vitamin D deficient and those who were vitamin D sufficient within each cohort. Association between serum vitamin D levels and PFS was evaluated with standard survival analysis techniques. Kaplan-Meier survival curves were estimated, and differences in survival time (between those who were vitamin D deficient and those who were sufficient) were assessed using the log-rank test. Cox proportional hazards regression was used to estimate the effects of serum vitamin D levels on PFS, controlling for prognostic index (Follicular Lymphoma International Prognostic Index [FLIPI] in LYSA; International Prognostic Index [IPI] in SWOG because S9800 predated FLIPI). In addition, we included body mass index (BMI), timing of baseline blood draw (by quarter), and latitude in the Cox proportional hazards model to control for potential confounding by these variables. Additional demographic and clinical factors associated with baseline serum vitamin D were also included in the cohort-specific Cox proportion hazards model to control for potential confounding effects. Cox regression, with inclusion of potential confounding variables, was also used to evaluate the association between vitamin D levels and overall survival (OS). Vitamin D was in addition modeled as both a continuous variable and a categorical variable by cohort-specific tertiles, within the cohort-specific Cox proportional hazards models. In exploratory analyses, the proportion of those achieving a complete response (CR)/unconfirmed CR among vitamin D–deficient patients was compared with the clinical response proportion among vitamin D–sufficient patients using the χ^2 statistic. Analyses were performed using SAS software (version 9.2; SAS Institute, Cary, NC).

RESULTS

Patient and Disease Characteristics

SWOG cohort. Figure 1A outlines patient inclusion in the SWOG analysis cohort. In summary, a total of 777 patients were enrolled onto three SWOG trials; 184 patients had baseline serum stored and available for 25(OH)D measurement (S9800, n = 28; S9911, n = 29; S0016, n = 127). One serum 25(OH)D assay failed. The final SWOG cohort for this analysis included 183 patients enrolled and treated in centers across the United States, as summarized in Table 1. The SWOG data set was closed for analysis on November 16, 2011; the patients in this analysis had been observed for a median of 5.4 years. No statistically significant differences were found between

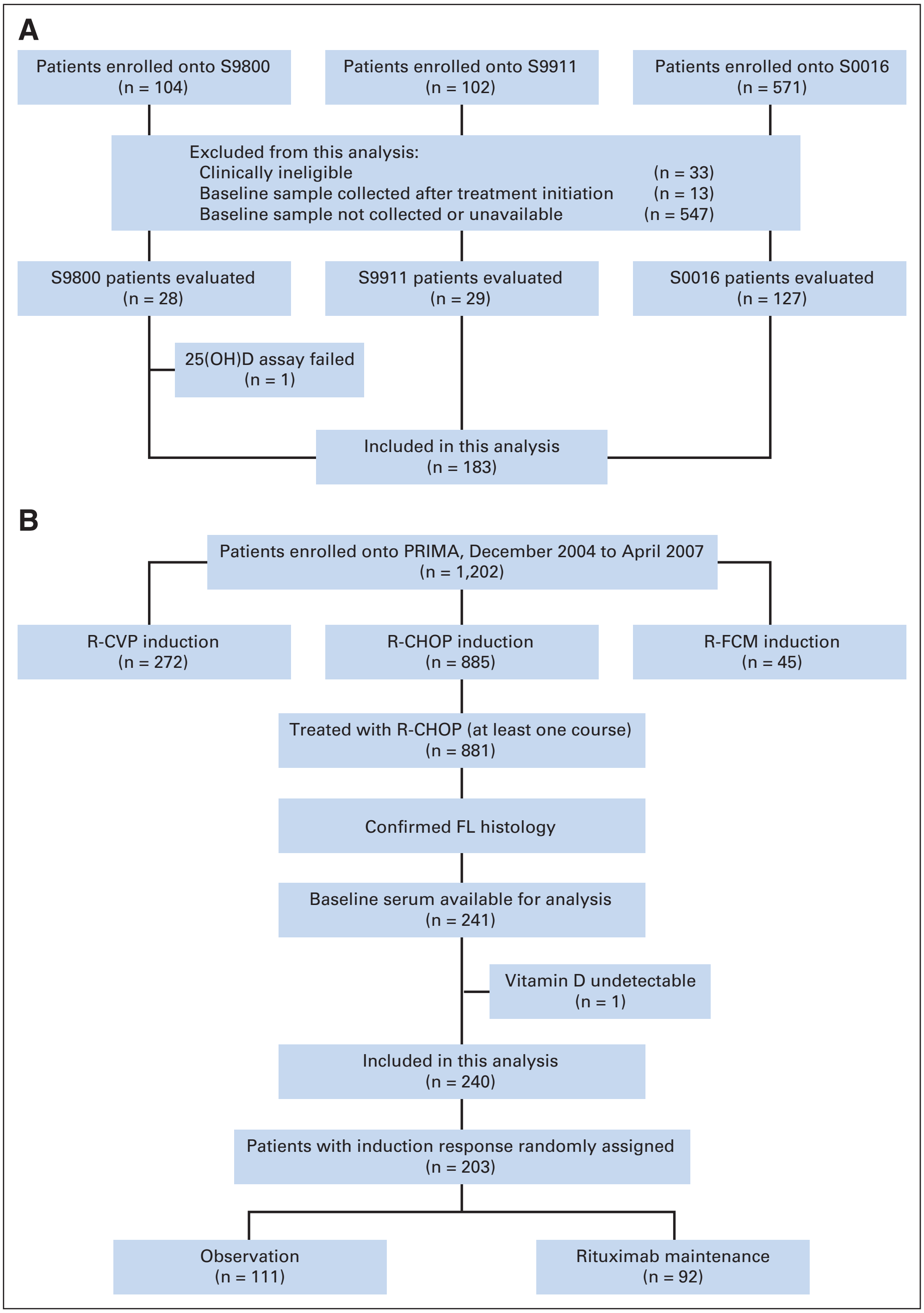

Fig 1. CONSORT diagram detailing source of patients included in (A) SWOG and (B) Lymphoma Study Association cohorts. 25(OH)D, 25-hydroxyvitamin D; FL, follicular lymphoma; PRIMA, Primary Rituximab and Maintenance; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab plus cyclophosphamide, vincristine, and prednisone; R-FCM, rituximab plus fludarabine, cyclophosphamide, and mitoxantrone.

SWOG patients included versus excluded from this analysis (Appendix Table A1, online only).

LYSA cohort. Figure 1B outlines patient inclusion in the LYSA cohort. In summary, a total of 1,202 patients were enrolled onto the parent PRIMA clinical trial, and the majority of these patients were treated with R-CHOP induction (n = 885). Of those, 241 patients had samples available for this study, and vitamin D was undetectable in one sample, leaving 240 patients. Only the patients with a response to induction therapy were randomly assigned to rituximab maintenance versus observation (n = 203); however, follow-up for PFS and OS events continued for the entire registered cohort. This LYSA cohort included patients primarily enrolled and treated in France (n = 219), Belgium (n = 15), and Australia (n = 6). The LYSA data set was closed for analysis on January 31, 2013, at which point the patients in this analysis had been observed for a median of 6.6 years. No statistically significant differences were found between LYSA patients included versus excluded from this analysis (Appendix Table A1, online only).

Table 1. Patient Demographic and Clinical Characteristics

	SWOG Cohort (n = 183)					LYSA Cohort (n = 240)				
	All Patients		25(OH)D < 20 ng/mL			All Patients		25(OH)D < 10 ng/mL		
Characteristic	No.	%	No.	%	P*	No.	%	No.	%	P*
Overall	183		28	15		240		60	25	
Sex					.34					**.009**
Male	100	55	13	13		127	53	23	18	
Female	83	45	15	18		113	47	37	33	
Age, years					.86					.876
≤ 60	130	71	19	15		154	64	39	25	
> 60	53	29	9	17		86	36	21	25	
Race					.33					
White	176	97	28	16						
Nonwhite	5	3	0	0						
BMI, kg/m^2					**.032**					**.010**
< 25	49	28	6	12		122	51	21	17	
25-30	73	41	7	10		77	32	23	30	
≥ 30	54	31	14	26		41	17	16	39	
Stage					.96					.235
II	58	32	9	16		21	9	3	14	
III/IV	125	68	19	15		219	91	57	26	
IPI Score					.91					
0-1	96	52	14	15						
2	59	32	10	17						
≥ 3	28	16	4	14						
Poor performance status										
2 (SWOG)	4	2	1	25	.59					
1-2 (LYSA)						88	34	30	37	**.003**
No. of extranodal sites					.84					.379
0-1	159	87	24	15		173	72	40	23	
≥ 2	24	13	4	17		67	28	20	30	
LDH					.28					.096
≤ ULN	139	76	19	14		164†	68	36†	22	
> ULN	44	24	9	20		75†	31	24†	32	
Hemoglobin < 120 g/L	12†	11				58	24	23	40	**.003**
No. of nodal sites ≥ 5	85†	24	14	16	.87	155	65	40	26	.697
FLIPI†					.33					.360
Low (≤ 1)	43	27	7	16		53	22	11	21	
Intermediate (2)	68	44	8	12		78	33	17	22	
High (≥ 3)	45	29	10	22		109	45	32	29	
B symptoms	‡		‡			83	35	23	28	.481
Elevated β_2-microglobulin	‡		‡			71†	30	15†	21	.330
Bone marrow involvement	‡		‡			138†	58	36†	26	.572
Quarter of enrollment					.22					**< .001**
First (January to March)	44	24	9	20		86	36	38	42	
Second (April to June)	42	23	9	21		59	25	14	24	
Third (July to September)	59	32	5	8		28	12	1	4	
Fourth (October to December)	38	21	5	13		51	21	6	12	
Induction therapy					.30					NA
CHOP-RIT	92	50	11	12						
R-CHOP	88	48	17	19				240	100	
R-CVP	—									
R-FCM	—									
CHOP	3	2								
Rituximab maintenance§					NA					.292
No	183	100				94	46	27	29	
Yes						112	54	25	22	

NOTE. Bold font indicates significance.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CHOP-RIT, cyclophosphamide, doxorubicin, vincristine, and prednisone plus iodine-131 tositumomab; FLIPI, Follicular Lymphoma International Prognostic Index; IPI, International Prognostic Index; LDH, lactate dehydrogenase; LYSA, Lymphoma Study Association; NA, not available; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab plus cyclophosphamide, vincristine, and prednisone; R-FCM, rituximab plus fludarabine, cyclophosphamide, and mitoxantrone; ULN, upper limit of normal.
*χ^2 test.
†Hemoglobin values available for 110 SWOG patients; data on number of nodal sites available for 156 SWOG patients; FLIPI values available for 156 SWOG patients; β_2-microglobulin available for 233 LYSA patients; LDH available for 239 LYSA patients; bone marrow involvement available/specified for 236 LYSA patients; quarter of enrollment available/specified for 224 LYSA patients.
‡Data unavailable for SWOG cohort.
§In LYSA cohort, 206 patients were randomly assigned to either rituximab maintenance or observation.

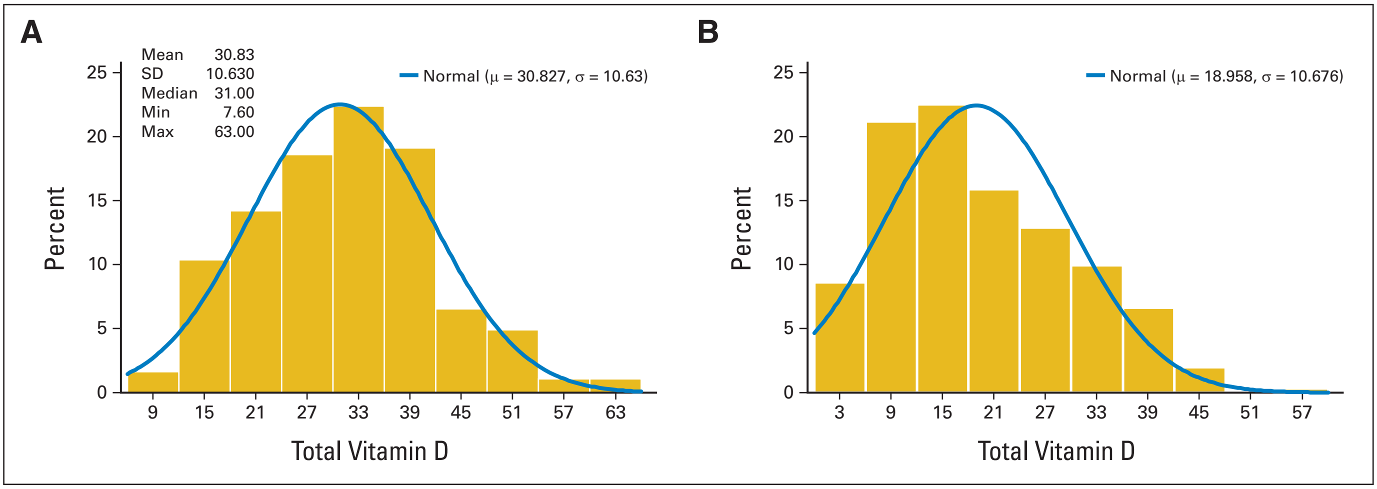

Fig 2. Serum 25-hydroxyvitamin D distribution in (A) SWOG and (B) Lymphoma Study Association cohorts. SD, standard deviation.

Vitamin D Distribution

SWOG cohort. Median serum 25(OH)D for the SWOG cohort was 31.0 ng/mL (standard deviation, 10.6 ng/mL). At the threshold of 25(OH)D < 20 ng/mL, 15% (n = 28) of this cohort was deficient. As expected, obesity (BMI > 30 kg/m^2; P = .032) and winter or spring enrollment (quarter 1 or 2 *v* 3 or 4; P = .08) were associated with vitamin D deficiency in this cohort (Table 1). Notably, there was no statistically significant difference in prevalence of vitamin D insufficiency between patients receiving CHOP-RIT (CHOP plus iodine-131 tositumomab) compared with R-CHOP induction therapy (P = .30).

LYSA cohort. Compared with that in the SWOG cohort (Fig 2A), the shape of the LYSA cohort serum 25(OH)D distribution (Fig 2B) was nearly identical; however, the entire distribution was notably shifted (median, 17.0 ng/mL; standard deviation, 10.6). At the threshold of 25(OH)D < 10 ng/mL, 25% (n = 60) of this cohort was vitamin deficient. Female sex (P = .009), obesity (BMI > 30 kg/m^2; P = .010), poor performance status (Eastern Cooperative Oncology Group 1 or 2; P = .003), hemoglobin < 120 g/L (P = .003), and winter or spring enrollment (quarter 1 or 2 *v* 3 or 4; P < .001) were associated with vitamin D deficiency in this cohort (Table 1). Notably, there was no statistically significant difference in baseline prevalence of vitamin D insufficiency between patients randomly assigned to observation as compared with those randomly assigned to rituximab maintenance (29% *v* 22%; P = .292).

Summary of Outcomes

SWOG cohort. After a median follow-up of 5.4 years, 44% of this cohort had experienced progression (n = 81; 100% of three patients who received CHOP alone; 51% of patients who received R-CHOP; 36% of patients who received CHOP-RIT; Table 2), and 18% of this cohort had died (n = 33; two of three patients who received CHOP alone; 19% of patients who received R-CHOP; 15% of patients who received CHOP-RIT). The majority of these patients died as a result of lymphoma (n = 22; 85% of 26 with known cause of death), and there was no association between vitamin D deficiency and cause of death in this cohort (χ^2 P = .55). There was no evidence of association between vitamin D deficiency and clinical response (odds ratio for CR, 1.14; 95% CI, 0.46 to 2.81; P = .78).

LYSA cohort. After a median follow-up of 6.6 years, 47% of this cohort had experienced progression (n = 112; 56% of 112 patients who received R-CHOP followed by observation; 33% of 94 patients

Table 2. Summary of Events

	SWOG Cohort (n = 183)						LYSA Cohort (n = 240)					
	PFS Events			Deaths			PFS Events			Deaths		
Treatment	No.	Total	Rate (%)	No.	Total	Rate (%)	No.	Total	Rate (%)	No.	Total	Rate (%)
CHOP alone	3	3	100	2	3	67	—			—		
R-CHOP, no rituximab maintenance	45	88	51	17	88	19	63	112	56	7	112	6
CHOP-RIT	33	92	36	14	92	15	—			—		
R-CHOP with rituximab maintenance	—			—			31	94	33	8	94	9
R-CHOP ± additional therapy*							18	34	53	10	34	29
Total	81	44		33	18		112	47		25	10	

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CHOP-RIT, cyclophosphamide, doxorubicin, vincristine, and prednisone plus iodine-131 tositumomab; LYSA, Lymphoma Study Association; PFS, progression-free survival; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone.

*In LYSA cohort, 240 patients received R-CHOP induction, but 34 were not randomly assigned (treatment failure, n = 10; R-CHOP toxicity, n = 4; major protocol violation, n = 10; consent withdrawal, n = 2; other reasons, n = 8). These patients were observed for event-free and overall survival outcomes, but they could have received additional therapy after R-CHOP induction.

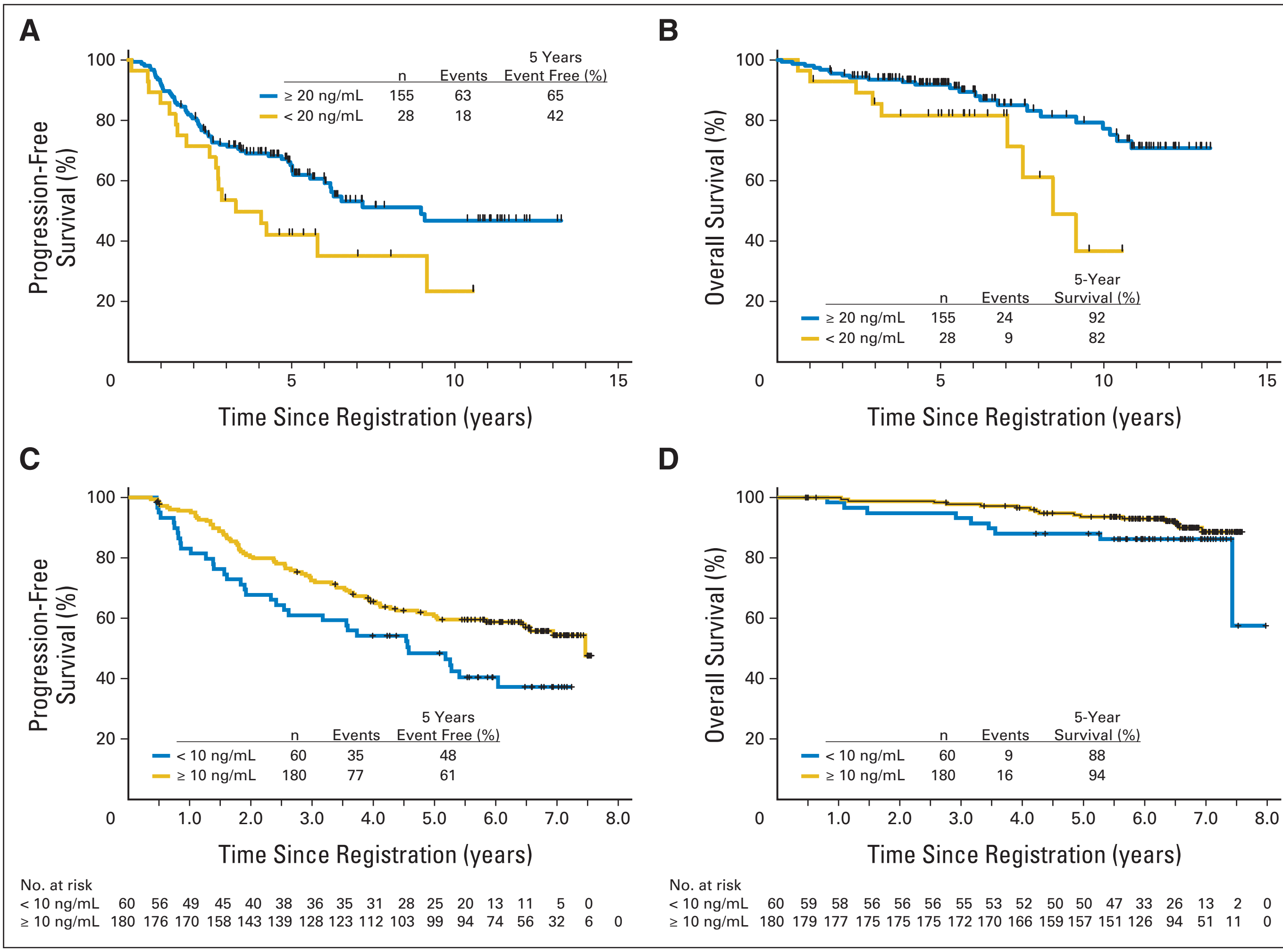

Fig 3. For (A, B) SWOG and (C, D) Lymphoma Study Association cohorts, (A, C) progression-free and (B, D) overall survival.

who received R-CHOP followed by rituximab maintenance; 53% of 34 patients who were not randomly assigned), and 10% of this cohort had died (n = 25; 6% of 112 patients who received R-CHOP followed by observation; 9% of 94 patients who received R-CHOP followed by rituximab maintenance; 29% of 34 patients who were not randomly assigned). The majority of these patients died as a result of lymphoma (n = 12; 48%), and there was no association between vitamin D deficiency and cause of death (χ^2 P = .601). There was no evidence of association between vitamin D deficiency and clinical response; the proportion of vitamin D–deficient and –sufficient patients achieving a CR/unconfirmed CR was 68% and 73%, respectively (χ^2 P = .508).

Association of Vitamin D Insufficiency With FL Outcomes

SWOG cohort. After a median follow-up of 5.4 years, vitamin D–deficient patients had significantly inferior PFS (hazard ratio [HR], 2.00; P = .011; Fig 3A) and OS (HR, 3.57; P = .003; Fig 3B) as compared with those with higher levels (Table 3). The magnitude and significance of these associations remained after analyses were stratified by treatment trial and adjusted for prognostic index (IPI), BMI, quarter of enrollment (3 or 4 *v* 1 or 2), latitude (≥ *v* < 35°N), and a quarter-by-latitude interaction term (PFS $HR_{adjusted}$, 1.97; P = .023; OS $HR_{adjusted}$, 4.16; P = .002). Sensitivity analyses were performed to exclude S0016 patients who were treated with CHOP alone (n = 3) and patients with performance status of 2 (n = 4) with no impact on effect estimates. Finally, when modeled as a continuous variable, 5-unit increases in vitamin D were not associated with PFS (HR, 0.94; P = .35) but were associated with OS (HR, 0.79; P = .04). Multivariable analysis of vitamin D by tertile confirmed that the lowest tertile of vitamin D was associated with the greater increase in risk of either progression or death, but neither result was significant.

LYSA cohort. After median follow-up of 6.6 years, vitamin D–deficient patients had significantly inferior PFS (HR, 1.66; P = .013; Fig 3C) but not OS (HR, 1.84; P = .14; Fig 3D) as compared with those with higher levels. The magnitude of these associations remained after analyses were adjusted for prognostic index (FLIPI), BMI, quarter of enrollment (3 or 4 *v* 1 or 2), latitude (Europe *v* Australia), hemoglobin, performance status, and sex (PFS $HR_{adjusted}$, 1.50; 95% CI, 0.93 to 2.47; P = .095; OS $HR_{adjusted}$, 1.92; 95% CI, 0.72 to 5.13; P = .192). Finally, when modeled as a continuous variable, 5-unit increases in vitamin D were not associated with PFS (HR, 0.95; P = .39) but were associated with OS (HR, 0.76; P = .059). Multivariable analysis of vitamin D by tertile confirmed that the lowest tertile of vitamin D was associated with the greater increase in risk of either

Table 3. Outcomes

Outcome	SWOG Cohort (n = 183)*						LYSA Cohort (R-CHOP induction only; n = 240)*					
		Events						Events				
	No.	No.	%	HR†	95% CI	*P*	No.	No.	%	HR†	95% CI	*P*
Crude PFS				2.02	1.18 to 3.45	.010				1.66	1.11 to 2.48	.013
Deficient	28	18	64				60	35	58			
Sufficient	155	63	41				180	77	43			
Crude OS				3.48	1.52 to 7.91	.003				1.84	0.81 to 4.18	.1427
Deficient	28	9	32				60	9	15			
Sufficient	155	24	15				180	16	9			
Adjusted PFS‡												
Deficient	27	17	61	1.97	1.10 to 3.53	.023	59	34	58	1.50	0.93 to 2.42	.0952
Sufficient	149	58	39				165	71	43			
Continuous (HR per 5-unit increase)	176	75	43	0.94	0.83 to 1.07	.35	224	105	47	0.95	0.85 to 1.07	.39
First tertile§	59	25	42	1.31	0.69 to 2.49	.402	88	49	47	1.53	0.86 to 2.71	.14
Second tertile	59	25	42	1.14	0.60 to 2.13	.69	72	31	30	0.96	0.55 to 1.69	.89
Adjusted OS‡												
Deficient	27	8	29	4.16	1.66 to 10.44	.002	59	9	15	1.92	0.72 to 5.13	.1924
Sufficient	149	22	14				165	15	9			
Continuous vitamin D (HR per 5-unit increase)	176	30	17	0.79	0.63 to 0.99	.04	224	24	11	0.76	0.56 to 1.01	.059
First tertile§	59	10	17	2.85	0.86 to 9.50	.089	88	14	59	5.32	1.11 to 25.63	.037
Second tertile	59	10	17	2.52	0.84 to 7.60	.10	72	7	29	2.76	0.57 to 13.34	.21

Abbreviations: BMI, body mass index; FLIPI, Follicular Lymphoma International Prognostic Index; HR, hazard ratio; IPI, International Prognostic Index; LYSA, Lymphoma Study Association; OS, overall survival; PFS, progression-free survival; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone.
*Vitamin D deficient: SWOG, < 20 ng/mL; LYSA, < 10 ng/mL.
†HR for deficient compared with sufficient.
‡SWOG HRs adjusted for: IPI, BMI, quarter of enrollment (3 or 4 *v* 1 or 2), latitude (≥ 35 *v* < 35), and quarter by latitude interaction. LYSA HRs adjusted for: FLIPI, BMI, quarter of enrollment (3 or 4 *v* 1 or 2), latitude (Europe *v* Australia), hemoglobin, performance status, and sex.
§Highest tertile is reference group.

progression or death, but only the association with OS reached significance (HR, 5.32; $P = .037$).

DISCUSSION

In this international collaborative study of patients with newly diagnosed FL similarly treated in the modern era and routinely observed for outcomes per clinical trial protocol, we report an association between low vitamin D levels and FL outcomes in two independent cohorts. The magnitude of the association may be among the strongest association reported to date of a pretherapy prognostic factor in FL.[22]

Our observations indicate that vitamin D insufficiency is relevant to outcomes in FL, and the impact on survival confirmed in two independent cohorts suggests that it is a robust predictive factor for patients with FL being treated with R-CHOP therapy. The differences in vitamin D distributions between these two international populations prevent us from drawing any conclusion with regard to optimal vitamin D levels in relation to FL prognosis, and indeed, the true threshold may differ between geographic regions and ethnic groups. The low serum vitamin D levels observed in the LYSA cohort were similar to those recently reported in a European cohort of patients with lymphoma (EPIC [European Prospective Investigation Into Cancer and Nutrition]),[23] a German cohort of patients with DLBCL,[7] and a Scottish cohort of patients with colorectal cancer.[24] Prior research in the Scottish cohort demonstrated the key influence of diet, environmental factors, and lifestyle on serum vitamin D levels,[25] and it is reasonable to hypothesize that such differences exist between the SWOG and LYSA cohorts. Furthermore, a recent large cohort study of community-dwelling Americans recently reported significantly lower levels of vitamin D among black patients as compared with white.[26] Differences in genetic polymorphisms between these two racial populations likely explain this observation.[27]

Our study is limited by the small proportion of parent cohorts (22% of SWOG; 29% of LYSA) eligible for inclusion in this analysis, and although their main characteristics did not differ from those observed in other patients not eligible, we acknowledge that results based on convenience samples such as ours are prone to biases that cannot be fixed with statistical analyses. However, the strength in our findings lies in the fact that associations between vitamin D insufficiency and FL outcomes were observed in two independent cohorts of newly diagnosed patients, all starting standard-of-care treatment with CHOP chemotherapy and an antibody, which was the most commonly used initial treatment for this disease in the United States.[28] In addition, the vitamin D levels were evaluated centrally in the same laboratory, using a gold-standard assay. The strength of our observed association in two independent cohorts of patients and the lack of multiple comparisons indicate our findings are unlikely secondary to chance. Mouse models have revealed that macrophages in particular are critical to antibody therapeutics in initiating antibody-dependent cellular cytotoxicity targeted against tumor cells labeled with antibody.[29] One hypothesized biologic mechanism for our finding is that the patients with insufficient vitamin D in our cohorts could have had impaired antitumor macrophage activity affecting their prognosis.[7]

Given the prolonged natural history of FL, we still have relatively few events with > 5.4 years of follow-up in each cohort. Although the adjusted PFS and OS estimates for the LYSA cohort did not reach statistical significance, it must be noted that there was limited power for multivariable modeling with many covariates, there were relatively few PFS events, and, in particular, there was a limited number of OS events in this cohort. However, estimated magnitude of the association between PFS and OS remains remarkably similar between the two cohorts. In addition, there is some evidence that the relationship between vitamin D levels and cancer outcomes may be confounded by additional health and lifestyle factors such as physical activity,[30] and this should be evaluated in future studies. Furthermore, vitamin D could be a surrogate marker for a healthy lifestyle. We feel there is potential clinical utility of 25(OH)D as a biomarker for improved outcomes in FL, regardless of whether there is a direct mechanism or a surrogate marker of better health.

In Europe and the United States, the most common presentation of FL is low tumor burden, where a period of observation without active treatment is often considered.[31,32] On the basis of our results, a study of vitamin D supplementation for patients with insufficiency could be considered. At present, this should only be done in the context of a clinical trial.

In summary, we are the first to our knowledge to report a strong association between low vitamin D levels, an easily measured and modifiable lifestyle factor, and FL outcomes in two independent cohorts. Together with the accumulating evidence to support the relevance of vitamin D in other lymphoma subtypes, these findings warrant further investigation. Future research is needed to determine whether vitamin D represents a proxy for health status or whether supplementation with vitamin D may be an attractive therapeutic option to ultimately change the natural history of this still incurable disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Low Serum Vitamin D Levels Are Associated With Inferior Survival in Follicular Lymphoma: A Prospective Evaluation in SWOG and LYSA Studies

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Appendix

Table A1. Comparison of Analysis Subset With Patients Excluded From Parent Cohorts

	SWOG Cohort (N = 777)					LYSA Cohort (PRIMA R-CHOP; N = 840)				
	Included (n = 183)		Excluded (n = 594)			Included (n = 240)		Excluded (n = 600)		
Characteristic	No.	%	No.	%	P*	No.	%	No.	%	P*
Sex					.87					.81
Male	100	55	330	56		127	53	312	52	
Female	83	45	264	44		113	47	288	48	
Age, years					.39					.55
≤ 60	130	71	441	74		154	64	398	66	
> 60	53	29	153	26		86	36	202	34	
Race					.1					
White	176	96	542	94						
Nonwhite	5	3	34	6						
BMI, kg/m^2					.65					.17
< 25	49	28	138	25		122	51	291	49	
25-30	73	41	230	41		77	32	229	38	
≥ 30	54	31	188	34		41	17	80	13	
Stage					.05					.49
II	3	2	27	5		21	9	62	10	
III/IV	180	98	564	95		219	91	538	90	
IPI score					.34					
0-1 (low)	96	52	304	51						
2 (high/intermediate)	59	32	224	38						
≥ 3 (high)	28	16	66	11						
Poor performance status										
2 (SWOG)	4	2	8	1	.42					
1-2 (LYSA)						82	34	213	36	.72
No. of extranodal sites					.77					
0-1	159	87	511	86		173	72	447	75	
≥ 2	24	13	83	14		67	28	153	26	
LDH					.96					.48
≤ ULN	139	76	450	76		164	69	395	66	
> ULN	44	24	144	24		75	31	203	34	
No. of nodal sites ≥ 5†	85	54	259	50	.34	137	57	331	55	.61
FLIPI†					.99					.43
Low (≤ 1)	43	27	141	27		53	22	132	22	
Intermediate (2)	68	44	228	44		78	33	221	37	
High (≥ 3)	45	29	148	29		109	45	246	41	
Elevated β_2-microglobulin†	‡	‡	‡	‡		118	51	296	49	.3
Bone marrow involvement†	‡	‡	‡	‡		138	59	323	54	.41
Time of enrollment†										
January to June	86	47	312	53		145	60	379	63	
July to December	97	53	275	46		79	33	221	37	
Induction therapy					.82					NA
CHOP-RIT	92	50	303	51						
R-CHOP	88	48	277	47		240	100	600	100	
R-CVP	—									
R-FCM	—									
CHOP	3	2	14	2						

(continued on following page)

Table A1. Comparison of Analysis Subset With Patients Excluded From Parent Cohorts (continued)

	SWOG Cohort (N = 777)					LYSA Cohort (PRIMA R-CHOP; N = 840)				
	Included (n = 183)		Excluded (n = 594)			Included (n = 240)		Excluded (n = 600)		
Characteristic	No.	%	No.	%	P*	No.	%	No.	%	P*
Rituximab maintenance§					NA					.42
No	183	100	594	100		34	14	71	12	
Yes						206	86	529	88	

Abbreviations: BMI, body mass index; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CHOP-RIT, cyclophosphamide, doxorubicin, vincristine, and prednisone plus iodine-131 tositumomab; FLIPI, Follicular Lymphoma International Prognostic Index; IPI, International Prognostic Index; LDH, lactate dehydrogenase; LYSA, Lymphoma Study Association; NA, not available; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab plus cyclophosphamide, vincristine, and prednisone; R-FCM, rituximab plus fludarabine, cyclophosphamide, and mitoxantrone; ULN, upper limit of normal.

*χ^2 test comparing patients included in versus excluded from analysis.

†Hemoglobin values available for 110 SWOG patients; data on number of nodal sites available for 156 SWOG patients; FLIPI values available for 156 SWOG patients; β_2-microglobulin available for 233 LYSA patients, LDH available for 239 LYSA patients; bone marrow involvement available/specified for 236 LYSA patients; time of enrollment available/specified for 224 LYSA patients.

‡Data unavailable for SWOG cohort.

§In LYSA cohort, 206 patients randomly assigned to either rituximab maintenance or observation.